# Synthesis of polypeptide based rod–coil block copolymers†

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**A bifunctional initiator was synthesized and used for a sequence of a nickel initiated polymerization of γ-benzyl-L-glutamate-*N*-carboxy anhydride and atom transfer radical polymerization of methyl methacrylate yielding a rod–coil block copolymer.**

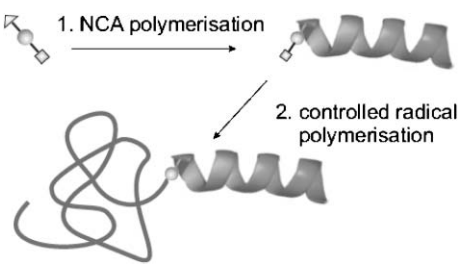
The integration of bio-inspired structure elements and classical polymer chemistry provides promising opportunities to design polymeric materials with unique solution and solid state properties. Examples are rod–coil type polymers comprising helical polypeptide and flexible hydrocarbon polymer blocks. Block copolymers of this architecture are of special interest from both functional and structural points of view. In contrast to coil–coil block copolymers, the self-assembly of rod–coil block copolymers is directed not only by the phase separation but also by the tendency of rigid segments to form anisotropic liquid crystalline domains. This competitive process can lead to supramolecular morphologies that are different from those commonly observed for block copolymers.[1–5]

The synthetic accessibility of well defined pure polypeptide based rod–coil block copolymers is challenging and until recently almost all polypeptide rod–coil copolymers reported in the literature were obtained by the macroinitiation of amino acid *N*-carboxy anhydrides (NCA) from amino terminated polymers.[1,5–7] Most of the applied macroinitiators were synthesized employing amino terminated azoinitiators, iniferters containing an amino group, or by subsequent tedious transformation of the polymer end-group into an amino group in polymer analogous reactions. This has the disadvantage that the often incomplete functionalization of the macroinitiator inevitably results in homopolymer impurity.[8] Moreover, side reactions in the subsequent NCA polymerization cause homopolypeptide impurities in the final product.[1,9] The macroinitiation of NCA employing terminal amino groups occurs *via* the "amine" mechanism,[10] *i.e.*, a nucleophilic attack of the amine on the NCA ring with subsequent ring-opening under release of $CO_2$. The formation of peptide homopolymer is preliminary caused by the transition from the desired propagation mechanism to the nucleophilic initiation by a deprotonated NCA anion (activated monomer mechanism).[10]

In order to gain control over the polymer structure and thus realize high block copolymer yield, minimizing all side reactions is of paramount importance. Significant progress with respect to the NCA polymerization was reported by Schlaad very recently. By *in-situ* reprotonation of the NCA during the polymerization, poly(styrene-*b*-*Z*-L-lysine) with exceptionally low polydispersity was obtained by macroinitiation from amino terminated polystyrene.[11,12] Another way to control the NCA polymerization was reported by Deming using a nickel complex as the initiating species yielding polypeptides with low polydispersity and controlled molecular weight.[13,14] Amino-terminated polyoctenamer and PMMA were successfully used as macroinitiators for block copolymers by this technique.[15]

In our laboratories we investigated a new synthetic concept to obtain polypeptide rod–coil block copolymers. In order to circumvent the limitations associated with the synthesis of amino terminated macroinitiators we apply a bifunctional initiator that allows both polymerizations to be conducted consecutively without intermediate polymer analogous functionalisation step. Moreover, to avoid the unfavourable macroinitiation of NCA, we inverse the standard reaction sequence by first synthesizing the polypeptide block followed by macroinitiation of the acrylic monomers (Scheme 1). We have chosen a nickel mediated NCA polymerization because this method has proven its feasibility in the polymerization of a variety of amino acid NCA's. Atom transfer radical polymerization (ATRP), on the other hand, was used for the synthesis of the flexible block due to its robustness and efficiency in macroinitiation.

The key to our approach is the bifunctional initiator **5**. It was synthesized as depicted in Scheme 2 with an overall yield of 23%. First alloc-L-leucin-*N*-hydroxysuccinimidyl ester **1**[15] was reacted with aminoethanol to yield alloc-L-leucin-(2-hydroxyethyl)amide **2**. Subsequently the ATRP initiator moiety α-bromoisobutyrate was introduced by esterification of the hydroxy group with acid bromide **3**, which yields the stable compound **4**. The latter can be converted into the initiating complex by reaction with the nickel cyclooctadiene complex ($Ni(COD)_2$) and phenanthroline as ligand. The initiating complex **5** can be isolated and stored under nitrogen. It contains an activated bromide group for ATRP and a Ni amido




**Scheme 1** Synthesis of rod–coil polypeptide block copolymers from a bifunctional initiator.

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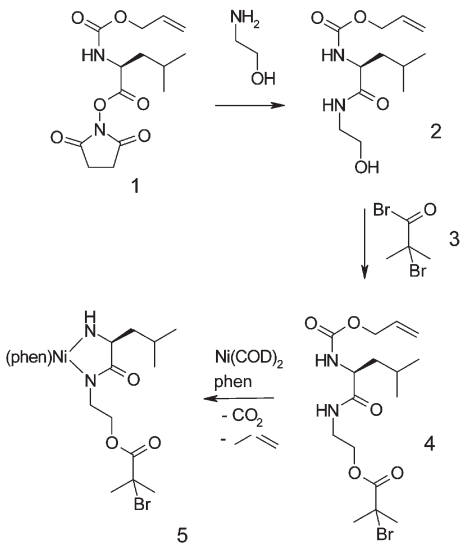

**Scheme 2** Synthesis of the bifunctional initiator.

amidate complex, which is the active initiator moiety for the Ni-mediated NCA polymerization. All intermediate structures were confirmed spectroscopically (see Supporting Information†). Unfortunately, structural analysis of **5** by NMR spectroscopy is not possible due to the paramagnetism of the Ni complex. Whilst the elemental ratios of C, O and N are in accordance with the proposed structure, a slight excess of Ni was found in the elemental analysis. This could not be completely removed by further purification. Similar deductions can be made from the data published by Deming.[16]

The feasibility of **5** for the NCA-polymerization was investigated for various monomer (γ-benzyl-L-glutamate NCA) to initiator ratios in DMF as solvent. We compared three different initiator batches in order to gain information on the effect of the residual Ni in **5** on the control of the polymerization. Inspection of Fig. 2 shows that an increasing monomer to initiator ratio leads to a reasonably linear increase of the molecular weight for each individual initiator batch as determined by SEC with a light scattering detector.

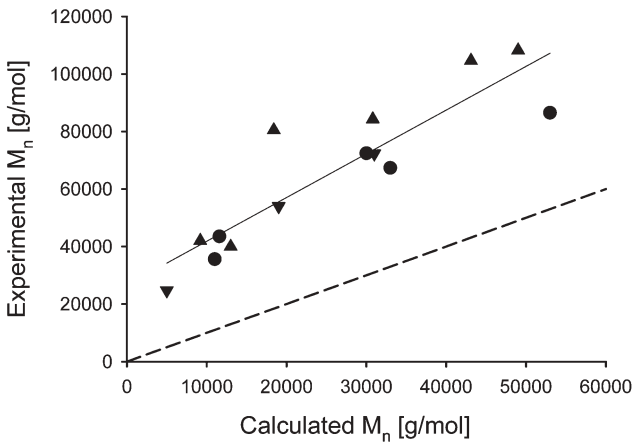


**Fig. 1** Comparison of calculated and obtained molecular weight for the poly(γ-benzylglutamate) polymerization employing **5** in DMF. The different symbols represent different initiator batches. The dashed line represents the expected molecular weight for 100% initiator efficiency.

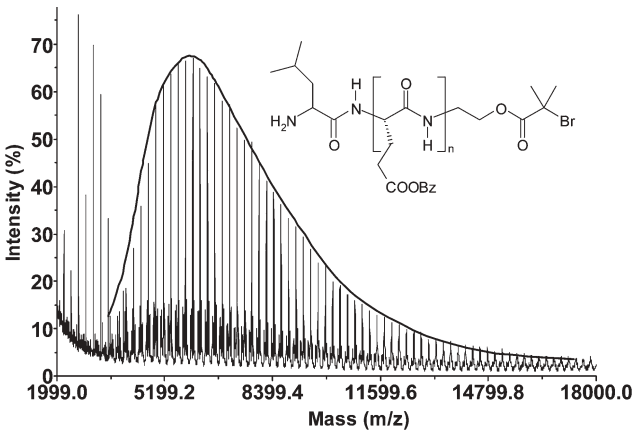


**Fig. 2** MALDI-TOF spectrum of PBLG initiated with **5**.

The experimental molecular weight obtained from every individual initiator batch is higher than the theoretical molecular weight. This suggests that the concentration of active initiator is lower than calculated from the monomer initiator ratio. We believe that this is due to the inactive impurities in the bifunctional catalyst. Similar effects have been reported by Deming for this type of initiator.[16] Nevertheless, the molecular weight and the polydispersity (1.2–1.4) of the PBLG block can be well controlled using initiator **5**.

Further evidence for the feasibility of the bifunctional initiator approach was obtained from MALDI-TOF analysis of the PBLG macroinitiator. MALDI-TOF spectra exhibit peaks with a mean distance corresponding to the monomer unit of PBLG (219 Da). Furthermore, the dominant molecular weight distribution comprises mass peaks corresponding to PBLG end-capped with both expected initiator fragments. This is in accordance with the proposed insertion mechanism of the metal mediated polymerisation and confirms the presence of the ATRP initiator group in the polypeptide. Evidence for the helical conformation of the polypeptide was obtained from data from IR spectroscopy (1652 $cm^{-1}$, 1548 $cm^{-1}$).

The PBLG macroinitiator was subsequently employed in an ATRP of MMA. Due to the low solubility of polypeptides in conventional ATRP solvents, suitable polymerization conditions had to be identified first. Recently, Aryes *et al.*,[17] Mei *et al.*[18] and Rettig *et al.*[19] reported the synthesis of block copolymers from sequenced oligo-peptides by ATRP in DMSO with CuBr/HMTETA or PMTETA, respectively. Although a reduced activity of the ATRP catalyst was observed by Rettig *et al.* due to copper complexation by the peptide amide groups, good control of molecular weight and polydispersity was obtained in this solvent. In our investigation, however, we could not achieve a controlled polymerization in DMSO at 90 °C. A control experiment replacing the PBLG macroinitiator by bromo-isobutyric acid ethyl ester under otherwise comparable conditions confirmed that this is not due to the PBLG. ATRP in DMSO has been shown to be very sensitive to the concentration of initiator, catalyst and monomer.[20] Due to the high molecular weight of the PBLG macroinitiators used in this investigation it was not possible to further increase the concentration. Better results were obtained using DMF as solvent, where a linear increase of the monomer conversion and thus the molecular weight as a function of time was observed (Fig. 3). We

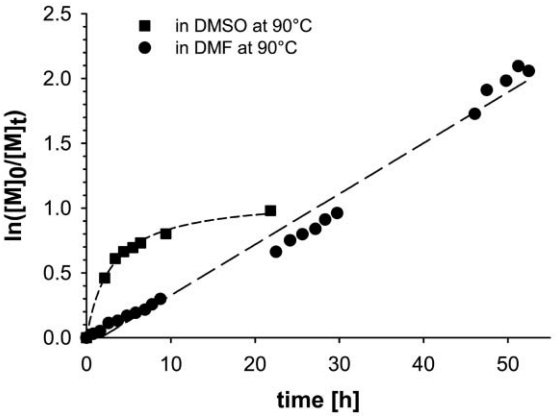


**Fig. 3** Conversion of MMA as a function of time for the polymerization employing a PBLG-macroinitiator. (33 vol% DMSO 803/1/4/2.5 [M]/[I]/[Cu$^{I}$Br]/[Ligand], 33 vol% DMF 811/1/1.1/1.3 M]/[I]/[Cu$^{I}$Br]/[Ligand]).

**Table 1** Results of the block copolymer synthesis by polymerization of MMA from PBLG macroinitiators of various degrees of polymerization ($P_n$): Comparison of $P_{n(theor.)}$ ([M]/[I] *conv.) and measured $P_n$ of the PMMA-block, MMA conversion is between 70 and 90% as determined by GC analysis

| Entry | $P_n$ (PBLG) | $P_n$ $(PMMA)_{theor.}$ | $P_n$ $(PMMA)_{exp.}$ [d] | $M_n$ [d] | PDI [d] |
|---|---|---|---|---|---|
| 1[a] | 110 | 1335 | 120 | 41 000 | 1.32 |
| 2[a] | 167 | 950 | 400 | 80 000 | 1.20 |
| 3[a] | 205 | 2465 | 130 | 67 000 | 1.39 |
| 4[b] | 94 | 705 | 275 | 51 800 | 1.36 |
| 5[c] | 304 | 620 | 300 | 110 000 | 1.22 |

[a] Catalyst: HMTETA/Cu(I)Br, solvent: DMF (33 vol%), 80 °C, time: 50 h. [b] Catalyst: HMTETA/Cu(I)Br, solvent: DMF (33 vol%), 90 °C, time: 50 h. [c] Catalyst: bis(2-pyridinal)ethylendiimine)/Cu(I)Br, solv.: DMF (33 vol%), 80 °C, time: 50 h. [d] Measured by GPC-MALLS (eluent DMF/LiBr).

tested several PBLG macroinitiators of different molecular weight in the block copolymer synthesis in DMF with CuBr/HMTETA (Table 1). In all experiments first order kinetics was observed. The SEC traces with THF as eluent show a clear shift of the peak maximum, with no evidence for non-reacted macroinitiator or PMMA homopolymer. The peak shift corresponds to a 1.3 fold increase of the molecular weight relative to polystyrene standards.

While these measurements clearly prove the successful synthesis of block copolymers, the exact molecular weights are difficult to determine using polystyrene calibration. The absolute molecular weights were therefore determined using SEC with DMF/LiBr as solvent and a light scattering detector. As can be seen from Table 1 these measurements clearly indicate an increase in molecular weight for all polymerizations. It has to be noted that SEC in DMF/LiBr probably underestimates the PMMA block because of its poor interaction with this solvent system and thus its small hydrodynamic volume fraction.[5]

In conclusion, a new approach for the synthesis of well defined polypeptide rod–coil polymers has been described combining nickel mediated NCA polymerization with ATRP. By employing a bifunctional initiator no intermediate polymer end-group modification is required. Moreover, the often unfavourable

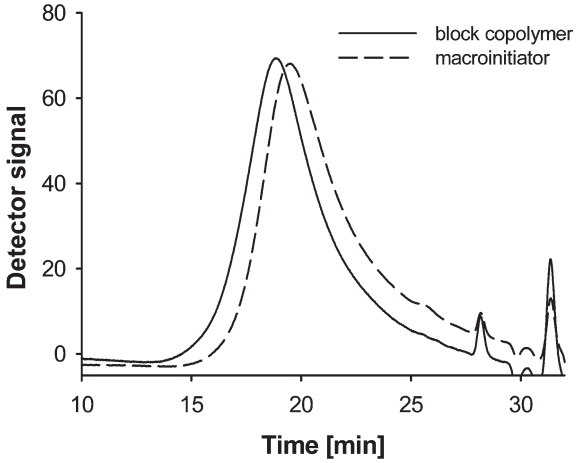


**Fig. 4** SEC traces of PBLG macroinitiator (entry 5 in Table 1) and PBLG-PMMA rod–coil block copolymer (eluent: THF).

macroinitiation of NCA was avoided by inversing the common reaction sequence, *i.e.*, synthesis of the polypeptide block first followed by ATRP.

The efficiency of the approach has successfully been shown for P(BLG-*b*-MMA) but we believe that it is universally applicable for the synthesis of a vide variety of polypeptide rod–coil block copolymers. Complimentary to other techniques this therefore increases the accessibility of a diverse range of interesting polypeptide rod–coil structures.

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